w/o, 28% with *TLR5-stop*) and a significant increase of 1yr TRM (25% to 39%, p 0.01) as well as overall TRM, which translated in decreased overall survival (51% vs 33%, p 0.002). The effect was strongest in the subgroup of younger and early stage pts receiving HLA-identical sibling SCT; in addition, combined analysis of *TLR5-stop* and *NOD2/CARD15* SNPs suggested a potential synergism, as 1 yr TRM was 21% in the absence of both SNPs, 32 and 33% in the presence of either *TLR5-stop* or *NOD2/CARD15* and 66% in the presence of both. In multivariate cox regression analysis of risk factors for 1yr TRM, older recipient age (HR1.5, p 0.04), female donor in a male recipient (HR 1.7, p 0.01) and presence of *TLR5-stop* (HR 1.6, p 0.05) and *NOD2/CARD15* SNPs (HR 1.8, p 0.004) in the recipient were confirmed as independent risk factors.

As both, *TLR- stop* and *NOD2/CARD15* SNPs have been associated with functional defects, our observations further support the concept of a major role of altered innate immune responses in the pathophysiology of GvHD associated complications following allogeneic SCT.

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IMMATURE DENDRITIC CELLS DOWNREGULATE GRAFT VERSUS HOST REACTIONS IN THE HUMAN SKIN EXPLANT MODEL AFTER CO-CULTURE WITH *IN VITRO* PUVA TREATED LYMPHOCYTES

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Extracorporeal Photopheresis (ECP) can be successfully used to treat steroid refractory Graft versus Host Disease (GvHD) after allogeneic hematopoietic stem cell transplantation. ECP is well tolerated and, different from conventional immunosuppressants, does not increase infection or relapse rates. One suggested scenario is that dendritic cells (iDC) might be modulated by ECP treated apoptotic lymphocytes and aquire a tolerogenic phenotype to downregulate inflammation in GvHD. We attempted to test this hypothesis using the human skin explant model.

Responder PBMCs and monocytes were derived from healthy donor buffy coats. Skin and stimulator cells were derived from patients after informed consent and ethical approval. A mixed lymphocyte reaction was set up. On day 4 responder cells were taken from the MLR. Apoptotic and dead cells were removed before treating half of the cells with *in vitro* PUVA (Psoralen, UVA) simulating ECP treatment. After 20h, the untreated or *in vitro* PUVA treated cells were added to monocyte derived immature DCs of responder origin and co-cultured overnight. The respective dendritic cells then were isolated via FACS sorting and added back to the ongoing MLR at a ratio of 1:10 (DC : MLR responder). Immature DCs were added at the same ratio to a third flask. Medium and Responder cells served as negative and positive control. After 48h 1×10^6 responder cells from the respective setup were given to skin sections derived from a dermabrasio. After 3 days the skin was routinally fixed and stained before evaluating the GvH grade using the Lerner scale.

Results: Immature DCs which had been in co-culture with *in vitro* PUVA treated PBMCs, but none of the controls, downregulated GvH reactions in the skin explant model from Grade III to grade I. MLR supernatants tested so far show reduced IFN-γ and TNF-α levels but no difference in IL-10 and IL-4 production. We are currently extending cytokine analysis and are further characterizing the apoptotic cells' influence on DCs in our model. FACS analysis of immature DCs after co-culture with apoptotic cells revealed upregulation of PDL-1 as a possible mechanism for lymphocyte suppression. Further investigation should reveal whether origin of the apoptotic cells or the method apoptosis is induced by are relevant for the observed effects.

Using the human skin explant model we could show that modulation of immature DCs by ECP/*in vitro* PUVA treated lymphocytes downregulates GvH reactions.

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SELECTIVE EXPANSION OF HUMAN REGULATORY ($CD4^+CD25^+CD127^{LOW}FOXP3^+$) CELLS TO HIGH PURITY BY INHIBITING EXPANSION OF $CD4^+CD25^+CD127^{HIGH}FOXP3^-$ CONVENTIONAL T CELLS WITH RAPAMYCIN

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$CD4^+CD25^+FOXP3^+$ natural occurring T regulatory (Treg) cells possess great therapeutic potential as adoptive cellular therapy for controlling acute GVHD. However, their clinical application is limited by the difficulty of obtaining sufficient numbers of $CD4^+CD25^+FOXP3^+$ cells. This study explored a strategy to expand human Treg in high purity and high numbers. By comparing expression of cell surface molecules on sorted $CD4^+CD25^{high}$ vs. $CD4^+CD25^-$ cells using mAb microarray, we identified CD127 was highly expressed in $CD4^+CD25^-$ and $CD4^+CD25^{Low}$ cells in human PBMC. Incorporating this new discovery, $CD4^+CD25^+$ cells were isolated by $CD25^+$ selection from PBMC, sorted into $CD4^+CD25^{High}CD127^{Low}$ and $CD4^+CD25^{low}CD127^{High}$ cells. FOXP3 were positive in 94% of $CD4^+CD25^{High}CD127^{Low}$ cells, and 5% in $CD4^+CD25^{low}CD127^{High}$ cells. The sorted cells were expanded with and without rapamycin in X-vivo medium containing IL-2 and anti-CD3/CD28 beads for a total of 21 days. In the absence of rapamycin, $CD4^+CD25^{High}CD127^{Low}$ cells expanded expanded 156 and 1560 folds, yet only 30% and 20% of expanded cells were positive for FOXP3 at day 14 and 21 respectively. In the presence of rapamycin $CD4^+CD25^{High}CD127^{Low}$ cells remain FOXP3+ in 96%, 60% and 56% at days 7, 14 and 21. However, the number of cells increased only 19, 36, and 21 folds at days 7, 14 and 21 respectively. To overcome the insufficient expansion of $CD4^+CD25^{High}CD127^{Low}$ cells in the presence of rapamycin, anti-CD3 mAb (OKT3) or anti-CD3/CD28 beads was added to the media at day 7 after the initial beads were removed and cultured until day 21. At day 14, 66% of the cells were FOXP3 positive with rapamycin and anti-CD3 and 80% for cells with rapamycin and ani-CD3/CD28 beads. The numbers of cells were expanded: 13 folds with rapamycin and anti-CD3, and 238 folds with rapamycin and ani-CD3/CD28 beads. At day 21, the numbers of cells were expanded: 34 folds with rapamycin and anti-CD3, and 2856 folds with rapamycin and ani-CD3/CD28 beads. In contrast, $CD4^+CD25^{low}CD127^{High}$ cells expanded in the same condition as controls had much lower percentages of FOXP3+ cells. **In summary**, in the presence of rapamycin, $CD4^+CD25^{High}CD127^{Low}FOXP3^+$ cells were preferentially expanded with IL-2 and anti-CD3/CD28 beads. Continued stimulation from anti-CD3/CD28 beads enhanced the expansion of $CD4^+CD25^{High}CD127^{Low}FOXP3^+$ cells. The suppressive function was positively correlated with the percentage of $FOXP3^+$ cells in the MLR culture.

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TREATMENT OF SEVERE OCULAR SURFACE DISEASE FROM OCULAR CHRONIC GRAFT-VERSUS-HOST DISEASE WITH A SCLERAL LEN PROSTHETIC DEVICE

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Purpose: The fluid-ventilated, gas-permeable scleral lens prosthetic device is an innovative treatment for ocular surface disease. Retrospective studies of patients with severe dry eye fitted with these lenses report mitigation of symptoms, improvement in function and quality of life, and healing of persistent epithelial defects of the corneal surface. This prospective study is designed to measure the impact of this device on visual function in patients with ocular chronic graft-versus-host disease (cGvHD) unresponsive to conventional therapy.

Methods: All patients referred to this institution for scleral lens consultation from January through June 2006 were administered the VFQ-25 at entry, and again 6 months later. The National Eye Institute sponsored the development of the VFQ-25 as a standardized, validated survey instrument to measure the dimensions of self-reported vision-targeted health status in persons who have

chronic eye diseases. Our patient database was sorted by diagnosis to identify the cGvHD subgroup in this cohort. The characteristics of this sub-group were extracted by database analysis and retrospective chart review. Initial VFQ-25 scores in this subgroup, their scores at 6-month follow-up, and change in score for those fitted with the scleral lenses will be reported.

Results: There were 16 patients with ocular surface disease from cGvHD seen for scleral lens consultation from January through June 2006. Demographic breakdown reveals M:F = 12:4, with age distribution as follows: 21-30, n =2; 31-40, n = 3; 41-50, n =3; 51-60, n=6; 61-70, n=2. Prior conventional therapy is reported. Mean baseline composite score on the VFQ-25 was 62 (range 35-91, n=16); scale is 0 – 100, with 100 representing highest level of function. Of these 16 patients, 13 were fitted with scleral lenses. Mean score for patients not fitted was 74 (n=3), whereas mean score for patients fitted was 59 (n=13). Preliminary analysis of data on patients who have reached the 6-month follow-up (n=6) reveals that each patient fitted had improvement of function. Mean score at 6 months is 70.5 (n=6). Mean change in score for the 6 patients for whom there is 6-month follow-up is +20.6 (n=6), with range from +2 to + 45.

Conclusion: The fluid-ventilated gas permeable scleral lens prosthetic device improves visual function in patients with ocular cGvHD unresponsive to conventional therapy.

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INCIDENCE AND OUTCOME OF CHRONIC GRAFT-VERSUS-HOST DISEASE (CGVHD) AFTER ALLOGENEIC STEM CELL TRANSPLANT (SCT) USING NATIONAL INSTITUTE OF HEALTH (NIH) CONSENSUS CRITERIA

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cGVHD, defined as GVHD after day 100, is common, with an impact on morbidity and survival. The limited/extensive cGVHD classification is not reproducible or prognostic for late non-relapse mortality (NRM). Recently the NIH consensus criteria were proposed, but the ability of these criteria to predict outcome of various types of cGVHD is unknown.

Pts undergoing their 1st SCT from 1/01 to 12/03 were studied. 110 pts alive beyond day 100 met criteria for the study. GVHD after day 100 was classified using NIH criteria into: persistent acute GVHD (aGVHD) (assigned at day 100), recurrent aGVHD, delayed aGVHD, classic cGVHD, overlap GVHD (all assigned at time of onset). Severity scores were assigned to pts with classic and overlap GVHD at onset and clinical worsening. Overall survival (OS) both from time of transplant and time of GVHD onset were measured.

37 (34%) had no GVHD and 73 (66%) pts had GVHD. OS was 44% vs. 66% (no GVHD vs.GVHD, P=0.026). Of 73 pts with GVHD, 14 (19%) had limited and 59 (80%) had extensive cGVHD. Pts with limited GVHD were reclassified as persistent aGVHD (7%), recurrent aGVHD (29%), and classic CGVHD (64%). Pts with extensive cGVHD were reclassified as persistent (3%), delayed (3%), recurrent (31%), classic chronic (37%), and overlap GVHD (26%). 31 (42%) had no subsequent clinical worsening and 42 (58%) had subsequent clinical worsening of GVHD. 65% of pts with classic cGVHD (22/31) had worsening compared to other types (20/42, 47%) (P=0.046). Severity scores increased in 12/31 pts (39%) at time of subsequent clinical worsening. OS of pts with various types of GVHD were significantly different (P<0.0001). This was more apparent when pts with any acute features of GVHD were compared with classic cGVHD (3-yr OS 47% vs. 66%, P=0.0015). This effect persisted when survival was measured from onset of GVHD (P=0.0336). Severity at onset or clinical worsening in pts with classic or overlap GVHD did not impact survival. The 3-yr NRM (with relapse as a competing risk) for the cohort was 21% and was not affected by the presence or absence of GVHD, or subtypes of GVHD. Significant variables using Cox model with time dependent covariates were any aGVHD feature after day 100 (HHR 5.27, P=0.0004), and extensive cGVHD (HR 0.28, P=0.0041).

The OS with different NIH subtypes after day 100 from SCT varies and is superior for pts with classic cGVHD. Global severity score, within the limits of our study had no prognostic value with respect to survival.

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RITUXIMAB RESPONSIVE REFRACTORY ACUTE GRAFT VERSUS HOST DISEASE

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The prognosis of steroid refractory acute graft-versus-host disease (SR-aGVHD) is poor with suboptimal responses to currently available agents. The role of B- lymphocytes in the pathogenesis of GVHD is unclear, but recent reports of successful responses to Rituximab in patients with cGvHD support the hypothesis that a coordinated B and T cell response is instrumental in cGVHD. The contribution of B-cells to the pathogenesis of aGVHD, however, is unknown. We now report 3 patients who received Rituximab with complete response of severe acute GVHD. The first patient (table-1) was a 51 year old female who developed severe transplant-associated thrombotic thrombocytopenic purpura (TA-TTP), manifesting as seizure, encephalopathy, cerebral edema, renal failure, fever and thrombocytopenia, which failed to respond to methyl prednisone and 26 sessions of plasmapharesis. Rituximab for refractory TTP was initiated on day + 58, while plasma exchange was continued. Following 3 doses of weekly Rituximab, there was resolution of TTP. At the time of initiation of Rituximab, the patient also had active grade III aGVHD that was refractory to steroids. GVHD improvement was noted from day +79 with complete resolution of aGVHD on day +95. During the course of Rituximab, steroids were progressively tapered. Beyond 100 days she had limited cGVHD involving skin that was well controlled on oral prednisone at 10 mg/day. 100% donor chimerism was present. Two additional patients with refractory aGVHD (Table 1) were then treated. Complete resolution of aGVHD occurred in 2 weeks, but did not begin until several days after stopping previous infliximab/daclizumab therapy. This observation of complete aGVHD responses to Rituximab in 3 patients requires confirmation in larger trial but suggests that B cells contribute to the pathogenesis of both acute and chronic GvHD.

Table-1: *Patient and disease characteristics of 3 patients with steroid refractory aGVHD*

Patient Age/Sex	Diagnosis Stage	Allogeneic transplant	Organ involved aGVHD grade	Previous GVHD Therapy	Timing/doses of Rituximab	aGVHD response	Outcome
51 F	DLBCL	Mel-Flu-Alem MUD/PBSCT	Skin III, Gut III, Grade III	Steroids (6.4 gm)	day +58 3 doses	Complete residual limited cGVHD	Died of sepsis on day +160
39 F	CML BC	CY/TBI MRD/ PBSCT	Skin III, Liver II, Gut III, Grade III	Steroids (3.6 gm) Infliximab x6	day +61 2 doses	Complete resolution	Alive in mCr
39 M	AML M-7	CY/TBI/Alem MUD/PBSCT	Liver II, Gut IV, Grade IV	Steroids (7.2 gm) Infliximab x5 Daclizumab x4	day +49 4 doses	Complete resolution	Alive in CR

Abbreviation: GVHD= graft versus host disease, aGVHD= acute graft versus host disease, cGVHD= chronic graft versus host disease, F= female, M= male, DLBCL= diffuse large B cell lymphoma, Mel= melphalan, Flu= Fludarabine, Alem= alemtuzumab, MUD= matched unrelated donor, PBSCT= peripheral blood stem cell transplant, CML= chronic myeloid leukemia, BC= blast crisis, CY= cyclophosphamide, TBI= total body irradiation, MRD= matched related donor, MTX= methotrexate, CR= complete remission, mCR= molecular complete remission.